

POSTER PRESENTATION

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Influence of PKG on insulin signalling and GSK3 phosphorylation in SH-SY5Y cells

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Background

Extracellular Amyloid- β (A β) plaques and intracellular neuro-fibrillary tangles (NFTs) of hyperphosphorylated Tau (τ) protein are considered to be the hallmarks of Alzheimer's disease (AD) [1]. A β is secreted due to the sequential cleavage of the amyloid β precursor protein (APP) by β - and γ - secretases (β cleavage) [1], whereas the intracellular signalling protein glycogen synthase kinase 3 (GSK3) has been implicated to cause τ - hyperphosphorylation leading to the formation of NFTs [2].

It has been shown earlier that the cleavage of APP by α - and γ - secretases (α - cleavage) is enhanced by insulin through the PI3K- Akt pathway [3]. GSK3 is a further downstream component of this pathway which has been shown to induce τ phosphorylation. Inhibition of GSK3 has also been shown to increase lysosomal biogenesis leading to autophagic degradation of APP [4].

Materials and methods

For the purposes of this study, human neuroblastoma (SH-SY5Y) cells were used. Cells expressing wtAPP, bovine cGMP dependent protein kinase 1-alpha (PKG1 α) and murine PKG2 were generated by lentiviral transduction and were stimulated with 200 μ M 8-pCPT-cGMP or/ and 1 μ M insulin for 15 min or 2 hrs. The cells were then lysed and the proteins analysed by Western Blotting.

Results

SH-SY5Y cells stably overexpressing APP, PKG1 α and PKG2 were used to analyze the crosstalk between the cGMP-PKG and insulin signalling cascades that was reported in brown adipose tissue [5]. As is known, upon insulin stimulation, the APP overexpressing cells showed a marked increase in the α -cleavage of APP with increased

secreted APP α levels (sAPP α). Analyzing the insulin pathway components in the cells overexpressing PKG (1 α or 2), a significant increase in phosphorylation of GSK3 was also seen when these cells were stimulated with cGMP, implying that PKG influences the downstream phosphorylation events in insulin signalling. While, PKG1 α overexpressing cells also showed a marked reduction in intracellular holoAPP levels with consequent reduction in extracellular sAPP α levels as well.

Conclusion

Our results suggest a possible crosstalk between cGMP-PKG and insulin signalling cascades. PKG1 α and PKG2 enhanced GSK3 phosphorylation upon cGMP stimulation, while PKG1 α affected levels of intracellular holoAPP thus reducing extracellular sAPP α levels.

Influence of PKG on GSK3 phosphorylation renders it as a viable and valuable target for AD therapeutics following a two-pronged approach; to reduce secreted A β levels by enhancing lysosomal biogenesis and simultaneous τ hyperphosphorylation reduction.

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